**ENGINEERING DECARBOXYLASES FOR CONSOLIDATED BIOPROCESSING AND MORE**

Volker Sieber, Technical University of Munich
Sieber@tum.de

Key Words: decarboxylase, thermostabilization, organic solvent, substrate promiscuity, biomass utilization

Decarboxylases have been widely applied for the production of chemical building blocks from biomass derivatives as well as chiral intermediates in organic synthesis.

First we have engineered different enzymes of this class (KDCA and PDC) for highly increased thermostability by combining rational design and random approaches. With melting points increased by almost 15 °C and half lifes above 70 °C prolonged up to several thousand fold this now opens up new possibilities for the production of alcohols such as ethanol, butanol or isobutanol from lignocellulose using thermophilic organisms in so called “consolidated bioprocessing” approaches, where lingo cellulose break-up is done simultaneously with product formation. In addition variants for higher tolerance towards denaturing, partially water miscible organic solvents were developed to be utilized e.g. for organic synthesis or in cell-free cascades like for the production of isobutanol from sugar.

Often, decarboxylases show broad substrate promiscuity, which can be of advantage but also a major challenge in fine chemical production. By further engineering the substrate specificity and modifying the promiscuity, it was possible to modulate the formation of desired chemicals with high selectivity. Based on this we have designed a network of artificial enzymatic cascade reactions towards production of bio-based building blocks utilizing decarboxylases as the modulating enzymes. With engineered variants, we demonstrate the conversion of industrial byproducts into several fine chemicals with high selectivity and yield. This result also sheds light on substrate recognition of the class of decarboxylases towards more-challenging substrates, e.g. bulkier side chains for broader applicability.